Supplementary figure legends:

**Fig. S1: NFATc2 deficiency is associated with less chronic inflammation in the DSS-colitis model.**

(A) NFATc2 deficient mice and wild-type mice were subjected to cycles of 2% DSS, as described in Methods. Miniendoscopic analysis was done during the second DSS cycle to determine colitis activity. Representative pictures for each group are shown. Inflammation could be observed in wild-type mice, whereas little or no inflammation signs were noted in NFATc2 knockout mice. (B) Scoring of DSS-colitis severity was performed in the second cycle of 2% DSS. Mean values +/- SEM are shown. Results are representative of four independent experiments (n=12).

**Fig. S2: Pro-inflammatory cytokine expression in NFATc2 knockout mice**

IFNγ and IL-1β mRNA levels were analysed using real-time PCR. (A) Relative expression of IFNγ normalised to β-actin in normal and tumor tissue of NFATc2 knockout and wild-type mice. (B) Real-time PCR analysis of IL-1β mRNA levels normalized to β-actin levels. Relative expression levels of IL-1β are shown in normal and tumor tissue from both strains. Significant differences are indicated.

**Fig. S3: Real time PCR analysis of cytokine production in the AOM/DSS experimental tumor model**

(A) Relative expression levels of IL-11 upon normalization to β-actin levels are demonstrated. Significant differences are indicated. (B) Relative expression of IL-22 was analysed upon normalization to β-actin levels and representative results are shown for both strains. Significant differences are indicated.
Fig. S4: Analysis of cytokine production in splenic T-cells and LPMC cells in the AOM/DSS experimental tumor model

(A) Cytokine production of splenic CD4$^+$ T-cells from wild-type and NFATc2 deficient mice. Cells were stimulated with antibodies against CD3 and CD28 for 48 hours, followed by analysis of culture supernatants using cytomix analysis kit. Significant differences are indicated. (B) LPMCs of wild-type and NFATc2 deficient mice were prepared, stimulated with PMA/Ionomycin, and supernatants were analyzed using cytomix analysis kit as described. Cytokine production of LPMC cells from both strains was determined. Data represent mean values from four different experiments and significant differences are indicated.

Fig. S5: NFATc2 and IL-6 expression in epithelial and myeloid cells

Cytospins preparation of isolated splenic cells and intestinal epithelial cells were analyzed by immunofluorescence staining for CD326(EpCAM), IL-6 and CD11c, as described in Methods. Cell nuclei were counterstained with Hoechst dye. (A) Cytospins of intestinal epithelial cells of wild-type and NFATc2 knockout mice were stained with a specific Anti-CD326 antibody, a specific epithelial marker, and an IL-6 antibody. Representative stainings are shown and negative control stainings are included. (B) Analysis of IL-6 expression in splenic myeloid cells from wild-type and NFATc2 knockout mice. Representative stainings of splenic cells from both species are shown. Arrows represent double positive cells.

Fig. S6: NFATc2 regulates COX2 and VEGF expression

(A) Real-time PCR analysis of COX2 expression in tumor tissue and normal tissue of wild-type and NFATc2 knockout mice expressed to house keeping gene $\beta$-actin. Significant differences are indicated. (B) Analysis of VEGF expression was done by real-time PCR in normal and tumor tissue from both strains related to house keeping gene $\beta$-actin. Graphics
reveal representative results from three independent experiments (mean values +/- SEM). Significant differences are indicated.

**Fig. S7: Analysis of the proliferation rate in normal and tumor tissue of wild-type and NFATc2 knockout mice**

Immunohistochemistry staining of crysections from normal and tumor tissue of wild-type and NFATc2 knockout mice was done with a specific antibody against the proliferation marker Ki-67. The nuclei were counterstained with Hoechst dye. Representative stainings of normal and tumor tissue as well as negative control stainings are shown. The expression of Ki-67 mRNA was analyzed by real-time PCR in normal tissue and tumor tissue of wild-type and NFATc2 deficient mice and normalized to 18sRNA levels. A significant increase of Ki-67 levels was noted in tumor tissue of wild-type mice as compared to NFATc2 knockout mice.

**Supplementary material and methods:**

**Isolation of lamina propria mononuclear cells**

Lamina propria mononuclear cells were isolated from freshly obtained colonic specimens using a modification of previously described techniques (32). In brief, the colon was washed in HBSS free of calcium and magnesium and incubated in HBSS containing 30 mM EDTA for 8 minutes at 37°C. After incubation, the epithelial cell layer was removed by intensive vortexing and passing through a 100-μm cell strainer. The tissue free of epithelial cells was cut in pieces and placed in 5 ml digestion solution containing 4% fetal calf serum, 0.5 mg/ml collagenase D, DNase I grade 2, and 50 U/ml dispase II (Roche). Digestion was performed by
incubating the pieces at 37°C for 20 minutes. After 20 minutes, the solution was passed through a 100-μm cell strainer (BD) and the digestion solution was inactivated by adding FCS at a 1:1 ratio. This procedure was repeated three times. The cells were cultured at a density of 2.5 x 10^6 /ml in IMDM supplemented with Streptomycin, Penicillin, Gentamycin and Amphotericin in a 37°C incubator with 5% CO₂. Stimulation of the cells was done by PMA and Ionomycin (Sigma) at a concentration of 1 μg/ml.

Preparation of cytospins
Intestinal epithelial cells were isolated from prepared colons with 30 mM EDTA in HBSS and shaking for 15 minutes at 37°C. Isolated spleen cells and intestinal epithelial cells from wild-type and NFATc2 knockout mice were isolated and stimulated with 1 μg/ml PMA and Ionomycin and 1.5μg Brefeldin A followed by an incubation step for 24 h at 37°C. Cells were washed twice in PBS and resuspended in 1x PBS. Slides were prepared in a Cytospin2-centrifuge (Shandon), loaded with 100 μl cell suspension and centrifuged at 300 rpm for 3 minutes. Slides were removed, dried for 2 hours and then incubated with specific antibodies against CD326 (BioLegend), followed with a secondary biotinylated antibody goat-anti rat and stained with streptavidin-Alexa488 reagent (Molecular Probes). Cytospins were blocked again and incubated with the Anti-IL-6 antibody (ebioscience) and stained by a secondary goat anti-rat antibody conjugated with Alexa555 (Molecular Probes). Double staining of CD11c and IL-6 were done with incubation of the cytospins with anti-CD11c (Invitrogen) and anti-IL-6 (ebioscience) antibodies followed by biotinylated goat anti-armenian hamster antibodies and goat-anti rat conjugated with Alexa555 antibodies (Molecular Probes) for IL-6. Finally, staining of CD11c was done by use of streptavidin conjugated Alexa488 dye (Molecular Probes). Stainings were analyzed by IX-70 fluorescence microscop (Olympus).

Immunofluorescence staining for the proliferation marker Ki-67
Cryosections from normal and tumor tissue of wt and NFATc2 KO mice were prepared and staining for the proliferation marker Ki-67 was done using a monoclonal rat-anti-mouse Ki-67 antibody (DAKO), followed by staining with a secondary goat-anti-rat antibody (Dianova) and as third step streptavidin-DyLight 549 reagent (Invitrogen) was used. Nuclei were stained with Hoechst 33342 dye (Life Technologies). Staining of the tissue sections was analyzed by IX70 fluorescence microscope (Olympus).

**Cytokine measurement**

Cell culture supernatants were taken 48 h after stimulation of splenic CD4\(^+\)-T-cells and LPMC. Cytokine concentration was measured by using commercially available mouse FlowCytomix systems (eBioscience).

**Analysis of cytokines in normal and tumor tissue by real-time PCR**

Total RNA was isolated from tumor and normal colonic tissue of wt and NFATc2 KO mice using the RNA micro kit (Machery & Nagel) according to manufacturer’s guidelines and transcribed into cDNA using Affinity Script Multi-Temp RT (Stratagene). Quantitative real-time PCR was performed with the QuantiTec SYBR Green Kit (peqlab). Analysis of the gene expression of IFN\(\gamma\), IL-1\(\beta\), IL-11, IL-22, COX2, VEGF and Ki-67 was performed by the use of gene specific primers (Qiagen) on a CFX-96 iCycler instrument (BioRad). House keeping gene \(\beta\)-actin were used to normalize gene expression results.